STRESS, PREDICTABILITY, AND ORAL FENTANYL
SELF-ADMINISTRATION IN FEMALE AND MALE RATS

1995

KLEIN

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES

4301 JONES BRIDGE ROAD
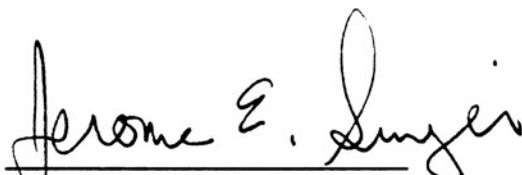BETHESDA, MARYLAND 20814-4799

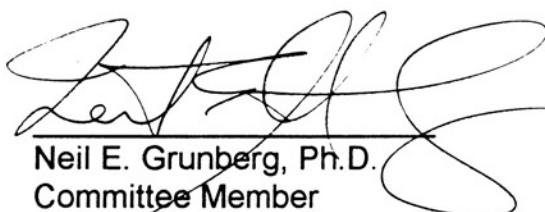## APPROVAL SHEET

Title of Thesis: "Stress, Predictability, and Oral Fentanyl Self-Administration in Female and Male Rats"

Name of Candidate: Laura Cousino Klein
                         Master of Science
                         March 9, 1995

Thesis and Abstract Approved:

_____   _____
Jerome E. Singer, Ph.D.   4 April '95
Committee Chairperson   Date

_____   _____
Neil E. Grunberg, Ph.D.   29 March 1995
Committee Member   Date

_____   _____
David S. Krantz, Ph.D.   4/5/95
Committee Member   Date

i

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"*Stress, Predictability, and Oral Fentanyl Self-*

Administration in Female and Male Rats"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.

Laura Cousino Klein
Laura Cousino Klein
Department of Medical and Clinical Psychology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Thesis: Stress, Predictability, and Oral Fentanyl Self-Administration

in Female and Male Rats

Laura Cousino Klein, Master of Science, 1995

Thesis directed by: Neil E. Grunberg, Ph.D.

Professor

Department of Medical and Clinical Psychology

This experiment examined the relationship between stress and drug self-administration (SA) in an animal model. Specifically, the effects of predictable and unpredictable footshock stress on oral fentanyl (50 µg/ml) consumption were examined in 12 female and 12 male Wistar rats using an operant conditioning paradigm. Female rats self-administered significantly greater amounts of fentanyl than did male rats and male rats exhibited more withdrawal behaviors following naloxone challenge. Predictability of a stressor was accompanied by significantly greater fentanyl SA, particularly for female rats. During relapse, animals exposed to predictable stress self-administered significantly less fentanyl during relapse stressor exposure than they did during the testing phase of the experiment. However, animals exposed to unpredictable stress self-administered similar amounts of fentanyl during relapse as they did during the testing phase. In addition, corticosterone levels were positively correlated with fentanyl SA.

Taken together, these results indicate that sex plays an important role in the initiation, maintenance, and relapse of drug-taking behavior by rats. In

iii

addition, these findings suggest that female rats either are more sensitive to the nonpharmacologic variable of predictability or are more sensitive to the drug-predictable stress interaction with regard to opiate SA. If these findings hold with clinical populations, then women and men may need different treatment approaches to opiate abuse.

Stress, Predictability, and Oral Fentanyl Self-Administration

in Female and Male Rats

by

Laura Cousino Klein

Thesis submitted to the Faculty of the

Department of Medical and Clinical Psychology

Graduate Program of the Uniformed Services University

of the Health Sciences in partial fulfillment

of the requirements for the degree of

Master of Science

1995

ACKNOWLEDGEMENTS

Dedicated to the Memory of

Sandra Jochum

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Stress and Drug Self-Administration

Clinical reports and observations suggest that there is a positive relationship between stress and opiate self-administration (SA). In addition, these reports suggest that stress might play an important role in drug relapse (Kosten, Rounsaville, & Kleber, 1986; O'Doherty, 1991; Shiffman & Wills, 1985; Whitehead, 1974). Unfortunately, limitations in the experimental design of these studies preclude a discussion of a causal relationship between stress and substance abuse (Hall, Havassy, & Wasserman, 1990; O'Doherty & Davies, 1987). Specifically, these studies are limited by small sample sizes, insufficient control groups, and inadequate appraisal of stress responses (i.e., psychological, physiological, and behavioral assessment). In addition, epidemiological studies provide correlational information that does not allow for causal explanations to be addressed.

Few studies have investigated the mechanisms that might mediate the stress-substance abuse relationship (Grunberg & Baum, 1985; Hall *et al.*, 1990; O'Doherty & Davies, 1987). Animal paradigms of drug SA provide an opportunity to examine the causal relationship between stress and substance abuse. Dib and colleagues (Dib & Duclaux, 1982; Dib, 1985) were the first investigators to conduct investigations of stress and opiate SA in rats. These investigations reported an increase in morphine self-administration by male rats during a footshock stressor. Morphine is a natural opium alkaloid that is used clinically for pain relief (Jaffe & Martin, 1990). Because drug SA was not evaluated either

1

before or after the footshock, it is possible that the observed increase in morphine SA by the subjects was a result of the analgesic effects of the opiate to decrease discomfort of the footshock rather than a stress-induced increase in responding for the reinforcing effects of the opiate.

Fentanyl is a synthetic opiate compound that is a phenylpiperidine (see Figure 1). It is primarily a μ-opiate receptor agonist that is approximately 80 times more potent than morphine and, like the other opiates, it is addictive (Jaffe & Martin, 1990). Because of its pharmacokinetic and pharmacodynamic properties, fentanyl is an excellent opiate compound to use in animal self-administration paradigms. Specifically, fentanyl is highly lipid soluble; therefore, it crosses the blood-brain barrier rapidly, regardless of the route of entry into the body. This synthetic opiate compound is quickly absorbed in the gastrointestinal tract and both its analgesic effects and euphoric effects are antagonized by opiate receptor antagonists such as naloxone. When dissolved in water, fentanyl hydrochloride (HCl) is less bitter-tasting than morphine and it is readily self-administered by rats (Shaham, Alvares, Nespor, & Grunberg, 1992).

Grunberg and colleagues conducted a series of experiments to build upon the Dib and Duclaux (1982; 1985) research but to avoid the ambiguity of the experimental designs. Specifically, these studies examined opioid SA following stressor exposure in order to evaluate drug SA that was not related to the analgesic property of the drugs. Shaham et al. (1992) reported that immobilization (IM) stress, prior to drug availability, increased home cage oral SA of both morphine and fentanyl over no-stress control conditions in male rats.

Shaham, Klein, Alvares, and Grunberg (1993) extended this work with a different stressor (mild, uncontrollable footshock stress) and in an operant self-administration paradigm. Shaham (1993) reported that drug conditioning factors also can play an important role in the stress-drug SA relationship. Taken together, these results suggest that a causal relationship exists between stress and opiate SA that is not related to the analgesic properties of these drugs. However, it still is not clear which mechanisms mediate the role that stress might play in drug SA. Shaham's (1993) report that conditioning alters stressors' (i.e., IM and electric footshock) effect on drug-taking behavior indicates that psychobiological variables are important in drug SA. In light of Shaham's (1993) report, other psychological variables might play a role in the relationship between stressor exposure and drug SA. One variable that has proved to be relevant in studies of stress is predictability.

Predictability of a Stressor

Predictability exists when an upcoming event or stimulus is signalled in advance either directly or by the context or timing of the event. In learning theory terms, predictability occurs when the probability of an unconditioned stimulus (UCS), given the presence or absence of a conditioned stimulus (CS), equals one (Miller & Matzel, 1989; Rescorla, 1966; Rescorla, 1967).

Predictability of an acute stressor may attenuate behavioral and physiological responses (Schulz, 1976; Staub, Tursky, & Schwartz, 1971; Weiss, 1970). For example, Glass and Singer (1972) reported a series of human laboratory studies which found that predictability of a noise stressor attenuated

the performance decrements that occurred after cessation of an acute unpredictable noise stressor . In contrast, under conditions of chronic stressor exposure, predictability potentiated behavioral, physiological, biochemical, and immunological stress responses (Abbott, Schoen, & Badia, 1984; Arthur, 1986; McKinnon, Weisse, Reynolds, Bowles, & Baum, 1989). This increase with repeated exposure to a stressor might reflect: (1) sensitization to the stressor; or (2) classical conditioning between the stimuli that predict the stressor and the stressor itself (Pavlov, 1906; Rescorla, 1966; Rescorla, 1967).

The present experiment was designed to examine effects of acute and then repeated stressor exposure (a form of chronic stress) on drug SA. Because sex differences in drug use and stress responses have been reported in the literature (Grunberg, Winders, & Wewers, 1991; Lex, 1991; Taylor, Harris, & Vogel, 1990), it also is important to examine the causal relationship between stress and drug SA in female and male rats.

Sex Differences in Drug Self-Administration

Taken together, Klein, Shaham, Alvares, and Grunberg (1993) and Shaham *et al.* (1993) reported differences between females and males in opiate SA following mild footshock stress. Male rats consistently increased opiate SA after this unpredictable, repeated, acute stressor, whereas only some of the female rats increased drug SA. The females rats were less water deprived during the training phase of the experiment and, consequently, may have been insufficiently trained to lever respond for the fentanyl solution. Because this study (Klein *et al.*, 1993) did not adjust water deprivation based on baseline

water consumption, it is impossible to determine the specific cause for differential

responding for the fentanyl solution. One purpose of the present experiment was

to examine further the causal relationship between predictable and unpredictable,

acute and chronic stress and drug SA in female and male rats.

OVERVIEW

The present experiment examined the effects of predictable versus unpredictable stress on fentanyl (50 µg/ml) self-administration (SA) in female and in male rats. Several hypotheses were tested. It was predicted that stress would increase drug SA over no-stress control conditions. It was hypothesized that, over time, predictable stress would increase drug SA over unpredictable stressor conditions. It also was hypothesized that sex differences in drug SA would occur. It was hypothesized further that drug-seeking behavior would diminish when the drug was no longer available and that stress would have no effect on this drug-seeking behavior. Further, it was predicted that relapse to fentanyl SA after an extinction phase would be altered by sex and stressor predictability.

In this experiment, fentanyl-HCl, in a concentration of 50 µg/ml dissolved in tap water, was used. The reinforcing effects of this drug concentration have been reported in the literature. This concentration of fentanyl is readily self-administered by male (Shaham *et al.*, 1993) and female (Klein *et al.*, 1993) rats and withdrawal behaviors can be evaluated by antagonism of the opioid system (e.g., naloxone). Therefore, in addition to the fentanyl, naloxone-HCl (1.5 mg/kg) was used to precipitate the withdrawal syndrome.

Throughout the experiment, animals were tested for oral SA (water or fentanyl) in operant conditioning chambers. Animals (12 male and 12 female Wistar rats) were assigned to predictable or unpredictable stress groups based on baseline lever responding for water. Using a repeated measures, within-subject design, animals were exposed to 3 seconds of either predictable or

6

unpredictable footshock stress (0.8 mA) over the course of 10 minutes prior to 30 minute access to the fentanyl solution. Animals were exposed to two cycles of stress and no-stress conditions (approximately 5 days for each phase).

Following this testing phase, water was substituted for the fentanyl solution to evaluate the reinforcing properties of the fentanyl. After this extinction phase, animals rested for seven days and then both the drug and the stressor were reinstated to evaluate drug relapse behavior. Drug relapse was evaluated under conditions of no-stress and stress.

Dependent variables included number of lever responses, amount of fentanyl self-administered, and latency to reinforcement. In addition, non-specific activity on the non-operative lever, daily body weight, and water consumption were recorded. Withdrawal behaviors were observed following intraperitoneal injection of naloxone on the last day of the first stress phase immediately following access to the drug. Biochemical measures were collected at the end of the experiment to evaluate the effects of predictable and unpredictable stress on plasma corticosterone.

MAJOR HYPOTHESES

Hypothesis 1. It was hypothesized that rats in the predictable stress condition would self-administer (SA) less fentanyl than would rats in the unpredictable stress condition during the beginning of the *Initiation Phase* of the experiment.

Rationale: It has been reported that predictability of a stressor can attenuate stress responses under acute conditions in humans (Glass & Singer, 1972; Schulz, 1976; Staub, Tursky, & Schwartz, 1971) and in animals (DeBoer, van der Gugten, & Slangen, 1989; Weiss, 1970). Therefore, animals in the unpredictable stress conditions should SA more fentanyl in response to the experience of higher stress.

Hypothesis 2. It was hypothesized that rats in the predictable stress condition would SA more fentanyl than would rats in the unpredictable stress condition throughout the *Testing Phase* of the experiment.

Rationale: It has been reported that, under conditions of repeated stressor exposure or chronic stress, predictability of a stressor may enhance stress responses (Abbott, Schoen, & Badia, 1984; Arthur, 1986; McKinnon, Weisse, Reynolds, Bowles, & Baum, 1989). Therefore, animals in the predictable stress conditions should SA more fentanyl in response to the experience of higher stress.

Hypothesis 3. It was hypothesized that, regardless of stressor condition, some female rats would SA lower amounts of the fentanyl solution than would male rats.

8

Rationale: Klein *et al.* (1993) reported differential responding for fentanyl solution in female rats. Amounts of fentanyl self-administered by female rats were equal to or less than those self-administered by males for a fentanyl solution (Shaham *et al.*, 1993).

Hypothesis 4. It was hypothesized that fentanyl SA would be higher following conditions of stress compared to no-stress conditions.

Rationale: Shaham *et al.* (1993) reported that male rats increased fentanyl self-administration following unpredictable stress compared to no-stress control conditions.

Hypothesis 5. It was hypothesized that female and male rats in both predictable and unpredictable stress conditions would exhibit greater withdrawal behaviors in response to naloxone challenge than would their non-stressed female and male counterparts.

Rationale: Shaham *et al.* (1992) and Shaham *et al.* (1993) reported that male rats exhibit higher withdrawal responses following opioid SA compared to male animals that have not been exposed to opiates. Klein *et al.* (1993) reported similar withdrawal effects in female rats that had self-administered fentanyl.

Hypothesis 6. It was hypothesized that stress groups would have similar behavioral indices of withdrawal following naloxone challenge.

Rationale: Research findings (Klein *et al.*, 1993; Shaham *et al.*, 1992; Shaham *et al.*, 1993) have reported that female and male rats exhibit higher withdrawal responses following fentanyl SA compared with female and male animals that have not been exposed to opiates.

Hypothesis 7. It was hypothesized that animals in the predictable stress condition would exhibit a greater biochemical stress response than would their counterparts in the unpredictable stress condition.

Rationale: Under chronic conditions of stressor exposure, predictability has been reported to potentiate responses to stress (Abbott, Schoen, & Badia, 1984; Arthur, 1986).

METHODS

Subjects

Subjects were 12 female and 12 male, drug-naive Wistar rats (Charles River Laboratories, Wilmington, MA). All rats were approximately 90 days old and weighed 220-250 g (females) or 340-380 g (males) at the beginning of the experiment. Animals were individually housed in polypropylene shoebox cages (35.6 cm x 15.2 cm x 20.3 cm), with wire grid flooring, at 23° C, 50% relative humidity, and a 12 hour light/dark cycle (lights on at 0700). Animals had continuous access to food (Agway ProLab Animal Diet RMH 3500) and tap water, except for the 40 minutes/day spent in the operant conditioning chambers. Body weight and water consumption were measured daily throughout the experiment.

An additional group (4 drug-naive animals; 2 males and 2 females) was housed under identical conditions but was not exposed to the experimental manipulations. This group served as a control group for assessment of naloxone-precipitated withdrawal behaviors and for assessment of the biochemical effects of the stressor.

Drugs

Fentanyl-hydrochloride (HCl) (NIDA, Baltimore, MD), in a concentration of 50 µg/ml dissolved in tap water, was used. Withdrawal syndrome was precipitated by intraperitoneal (IP) injection of 1.5 mg/kg Naloxone-HCl (DuPont Pharmaceutical). Naloxone-HCl was suspended in 0.86% NaCl solution in a

concentration of 0.4 mg/ml.

Procedure

Animals were tested for oral self-administration (SA) in one of four sound-attenuated, operant conditioning chambers (ENV-001, Med Associates Inc., East Fairfield, VT). Each chamber was equipped with two levers (located 7 cm above the grid floor) and two 65 ml liquid dispensers (ENV-201). The left lever (operative) was initialized to administer 0.1 ml of solution after a predetermined number of presses, while right lever (non-operative) presses had no consequences and served as a measure of non-specific activity. Throughout the experiment, a 0.1 sec white noise burst was turned on every time a subject met the schedule requirement for a reinforcement. The operant chambers were connected to a power supply (SG600/C) and were run by a computer using MED-PC Medstate Notation (Tatham & Zurn, 1989), programmed in Turbo Pascal®. Figure 2 presents a timeline of the experiment.

Training Phase. On day 1, rats were allowed to lever press for water for 30 minutes under a fixed ratio (FR) 1 schedule following 48 h water deprivation. Then, throughout the *Training Phase*, animals were provided with 18% of their previous daily water intake (approximately 10 ml/day for males and 5 ml/day for females) in home cages and could lever press for water 30 min/day in the operant chambers. During this phase, schedule requirements were increased gradually from FR-1 to FR-4 and then to a progressive ratio (PR) schedule with a dwell of 3. That is, the schedule requirements were increased by a fixed ratio of one after every third reinforcement was given. A PR schedule was used to

provide a more sensitive index of the reinforcing efficacy of fentanyl (Depoortere, Li, Lane, & Emmett-Oglesby, 1993; Roberts, Bennett, & Vickers, 1989) than the FR schedules used in previous studies (Shaham et al., 1993). This *Training Phase* lasted 15 days. When responding on the operative lever stabilized (i.e., consistent number of responses for each subject for 3 consecutive days), the *Initiation Phase* began.

Initiation Phase. Following training, fentanyl-HCl solution (50 μg/ml) was substituted for water and the animals were assigned to either predictable stress or unpredictable stress conditions ensuring that baseline responding for water on the PR days was comparable. Animals (n=12; 6 males, 6 females) in the PS group received a 1 second presentation of an 86 dBA tone (Davis & Levine, 1982) immediately before each shock to signal that the stressor would occur. Animals in the UPS group received a similar 1 second tone, but it was presented (X = every 40 s; range 10-70 s) independently of the shock stimulus and, therefore, did not predict the onset of the stressor. Mild, constant-current, intermittent, inescapable, predictable or unpredictable, electric footshock (0.8 mA) was delivered under a variable interval schedule. Footshock was delivered through a scrambler to the grid floor of the operant conditioning chamber and was administered every 40 seconds on average (range 10-70 s) for 200 msec bursts. This amount and duration of shock has been used in previous research as a mild stressor (Shaham et al., 1993). The chambers were illuminated by a houselight as a discriminitive stimulus over a 10 minute period prior to the 30 minute drug SA period. The houselight was off during the drug SA period.

Throughout the *Initiation Phase*, the volume of water available to the animals in their home cage was increased by 10-15 ml/week (males) or 3-6 ml/week (females) until all animals consumed less water than the amount available to them. This criterion was achieved by all animals within 28 days.

Testing Phase. This phase included 4 cycles that lasted 5 days each: stress, no-stress, a second exposure to stress, and a second cycle of no-stress. During each cycle, animals could lever press for fentanyl under a PR schedule with a dwell of 2. The stress cycles were conducted as described for the *Initiation Phase*. Specifically, animals received 3 seconds of either predictable or unpredictable footshock over the course of 10 minutes prior to the 30 minute drug SA period. For the no-stress cycles, animals were placed in the operant chambers for 10 min prior to the 30 min drug SA period while the shock and houselight were turned off. The stress cycles were conducted as described for the *Initiation Phase*. Throughout this phase, water (500 ml) was continuously available in the home cage.

On the last day of the first stress cycle (day 5), after the 40-minute test session, naloxone (1.5 mg/kg) was injected IP into each animal, and subjects were transferred to their home cages. Assessment of withdrawal behaviors (wet-dog shakes, diarrhea, mouthing and teeth chattering, ptosis, excessive grooming, abnormal posture) began 5 minutes after injection and lasted for 15 minutes. This procedure was based on previous reports (Linseman, 1977) and has proved to be a highly reliable procedure for opioid withdrawal assessment (Shaham *et al.*, 1992; Shaham *et al.*, 1993) (inter-rater reliability coefficient: Pearson's

product-moment correlation = +0.96). This withdrawal assessment procedure also was followed for the four animals not exposed to the operant conditioning paradigm. This *Testing Phase* lasted for 20 days.

Extinction Phase. Following this drug testing phase, lever responding behavior was extinguished by substituting water for the fentanyl solution in the operant chambers. This *Extinction Phase* lasted for 20 days and animals were tested for lever responding for water under conditions of stress (13 days), no-stress (5 days), and a second exposure to stress (2 days).

Relapse Phase. At the end of the *Extinction Phase*, animals were left in their home cages with continuous access to food and water for 1 week. Subsequently, animals again were tested for fentanyl SA in the operant chambers under conditions of no-stress (4 days) and then stress (5 days) to examine relapse drug SA behavior.

Rest Phase. Animals were left in their home cages for 3 weeks with continuous access to food and water.

Biochemical Assessment of Stress. After this 3 week latency, animals were exposed to the stressor, with or without predictability, for 10 minutes a day and then immediately were returned to their home cages. This procedure lasted for 5 days. On the last day (day 5), animals were taken out of the operant chambers and, 15 minutes later (Seggie & Brown, 1975), were decapitated without anesthesia. Trunk blood was collected in 5 ml collection tubes that had been treated with ethylenediamine tetra-acetic acid (EDTA). Samples then were centrifuged for 20 minutes at 1500 x g at 4° C, and plasma was frozen at -70° C

for later measurement of corticosterone by radioimmunoassay (RIA; ICN Biomedical, Palo Alto, California). Corticosterone is a sensitive and commonly used marker of stress (Kant *et al.*,1983; Seggie & Brown, 1975).

Statistical Analyses

Overall, this experiment used a mixed factorial design. The between-subjects design was a 2 (sex; female/male) X 2 (stressor condition; predictable/unpredictable) and was used to examine the effects of stress on fentanyl self-administration. Between-subjects dependent variables evaluated by univariate and multivariate ANOVAs were: fentanyl consumption, operant and home cage water consumption, body weight, withdrawal scores following naloxone challenge, and plasma corticosterone levels. In addition to the 4 experimental groups, a control group (N=4; 2 males and 2 females) was used to compare no-stress control conditions to stress (i.e., predictable and unpredictable males and females) conditions on withdrawal scores and plasma corticosterone measures using analyses of variance.

The within-subject analyses for this experiment included the 4 experimental stress groups (i.e., predictable and unpredictable females and males) evaluated across the 5 phases of the experiment: initiation, training, testing, extinction, and relapse. Fentanyl self-administration amounts were computed for each animal for each session. Specifically, the number of reinforcers that each animal received per session was multiplied by the amount of liquid dispensed per reinforcement (0.1 ml) and by the concentration of the fentanyl solution (50 µg of fentanyl-HCl/ml of water). This product then was

divided by the animal's body weight (kg). Repeated measures ANOVA were used to evaluate the effects of stress on lever responding and fentanyl solution consumption (mg/kg) during each phase of the experiment (i.e., initiation, training, testing, extinction, relapse). Water consumption in the home cage, body weight, responding on the non-operative lever, and latency to the first reinforcement also were evaluated by repeated measures ANOVA.

Regression analyses were used to evaluate: (1) the relationship between fentanyl SA and withdrawal responses following naloxone challenge; and (2) the relationship between fentanyl SA and plasma corticosterone levels and included all subjects. All significance tests were two-tailed and were evaluated at an alpha level of 0.05.

Results

Overview

All animals were included in the analyses because each had sufficiently learned to respond on the operative (left) lever in order to obtain the reinforcer (water or fentanyl solution). A Student's *t*-test on lever responding behavior during the first stress phase of testing revealed that operative (left) lever responding was significantly greater than non-operative (right) lever responding $[t(46) = 6.26, p < .05]$ overall and for each experimental subject.

Initiation Phase

The first 13 days of the initiation phase were selected to examine the acute effects of unpredictable and predictable stress on drug self-administration.

To ensure that animals within the same sex were equally water deprived between experimental conditions (i.e., predictable, unpredictable), separate univariate ANOVAs were conducted separately for each sex on homecage water consumption and body weight (kg). During this drug initiation phase of the experiment, there were no differences in water consumption between males in the unpredictable stress condition and males in the predictable stress condition [$F_{(1,10)} = 4.4$, n.s.]. This finding indicates that these animals were similarly water deprived and that any differences in drug self-administration among the males between the 2 stress groups could not be attributed to differences in water deprivation. In addition, there were no differences in water consumption between females in the unpredictable and females in the predictable stress conditions [$F_{(1,10)} = 2.8$, n.s.], indicating that these two groups also were similarly water deprived. Also, there were no significant differences in body weight between males in the unpredictable and males in the predictable stressor conditions [$F_{(1,10)} = 3.67$, n.s.]. Females in the unpredictable and in the predictable stressor conditions also weighed the same [$F<1$].

A repeated-measures ANOVA on amount of fentanyl consumed (mg/kg) across the first 13 days of drug initiation revealed a significant time effect [$F_{(12,240)} = 10.07$, $p < .05$], a sex by time interaction [$F_{(12,240)} = 2.96$, $p < .05$], and a stress group by time interaction [$F_{(12,240)} = 1.38$, $p < .05$]. This stress group by time interaction did not hold for males but approached significance for females [$F_{(12,120)} = 1.72$, $p = .07$]. The sex by time interaction was true for the unpredictable stress group [$F_{(12,120)} = 1.93$, $p < .05$] and for the predictable

stress group [F (12,120) = 2.40, p < .01].

In order to determine the time point during initiation that the experimental groups (unpredictable/predictable, female/male) began to differ from one another, ANOVAs were conducted at each time point for the amount of drug self-administration. Figure 3 presents the average amount of fentanyl consumed (mg/kg) by the four experimental groups on the first, second, and third days of initial drug exposure. There were no differences between groups on the first or on the second day of exposure to the fentanyl solution. By the third day, there was a significant main effect for sex. Specifically, females self-administered significantly more fentanyl than did the males [F (1,20)=13.86, p < .05]. This significant sex effect persisted from day 3 throughout the remainder of the study (see Table 2). No main effects or interactions were found for stressor condition through day 27 of initiation.

Testing Phase

Once the testing phase began, there was a significant main effect for predictability [F (1,20) = 6.62, p < .05], with predictable greater than unpredictable, and a significant main effect for sex [F (1,20) = 19.79, p < .05], with females greater than males, for the amount of fentanyl that was self-administered. A repeated-measures ANOVA revealed a significant effect for time across the four phases of the testing phase [F (3,60) = 4.29, p < .05].

Figures 4, 5, 6, and 7 present fentanyl SA during the first stress, no-stress, second stress, and second no-stress testing conditions, respectively. ANOVAs were conducted at each time point for fentanyl SA among experimental groups to

examine the effects of stress and of no-stress on fentanyl self-administration. On the first day of testing during the first stress phase, animals in the predictable stress condition self-administered more fentanyl than did animals in the unpredictable stress condition [F (1,20) = 6.62, p < .05]. During the first stress phase (days 50-54), females self-administered nearly twice as much fentanyl (X=0.24 mg/kg) as did the males (X=0.13 mg/kg) [F (1,20) = 18.2, p < .05] and the predictably stressed animals self-administered significantly more fentanyl than did the unpredictably stressed animals [F (1,20) = 7.26, p < .05] (see Figure 4). The main effects for sex [F (1,20) = 15.45, p < .05] and predictability [F (1,20) = 4.99, p < .05] remained during the no-stress phase (see Figure 5) and during the second stress phase [F (1,20) = 18.52, p < .05 and F (1,20) = 5.28, p < .05, respectively] (see Figure 6). During the second no-stress phase (see Figure 7), the main effect for sex continued [F (1,20) = 16.72, p < .05], but the main effect for predictability only approached significance [F (1,20) = 3.35, p = .08].

There were no significant differences between experimental groups in number of lever responses on the non-operative (right) lever. There were no differences in home cage water consumption between the unpredictable and predictable stress groups or between males and females at any time during testing. In addition, there were no significant differences in body weight between the predictable and the unpredictable stress groups at any point during the experiment. However, females weighed significantly less than did the males throughout the testing phase of the experiment [F (1,20) = 2.83, p < .05].

Withdrawal

To evaluate whether or not female and male rats in both predictable and unpredictable stress conditions exhibited greater withdrawal behaviors in response to naloxone challenge than did non-stressed female and male rats, experimental groups were collapsed across stress conditions to form a stress group (n=24) that was then compared with the non-stressed control group (n=4). Observation scores across the six categories of withdrawal symptoms were added together from each observer to compute an overall composite withdrawal score for each subject. Total withdrawal scores ranged from 2 to 37. The variances within the stress and non-stressed control groups were small enough to allow this comparison to be made without violating the assumption of homogeneity of variance. Therefore, an ANOVA was computed for total withdrawal score between stress and no-stress groups.

Figure 8 presents the mean withdrawal score for males and for females in each experimental group (i.e., unpredictable stress (N=12), predictable stress (N=12), and no-stress control (N=4)] following an IP injection of 1.5 mg/kg of naloxone after either fentanyl (predictable and unpredictable stress groups) or no fentanyl (control group) SA on the last day of the first stress testing phase. Subjects that had self-administered fentanyl displayed significantly greater withdrawal behaviors than did animals that did not have previous access to the fentanyl solution [F (2,24) = 15.95, p < .05]. Also, males exhibited greater withdrawal than did females [F (2,24) = 5.42, p < .05]. There were no differences among groups (predictable, unpredictable, control) on the dosage of naloxone

that was administered [F (2,22) = 1.47, n.s.].

Despite the finding that predictable animals self-administered higher amounts of fentanyl (mg/kg) than did unpredictable animals, there were no significant differences between the two groups for withdrawal scores. In the stress group, there was a main effect for the amount of fentanyl (mg/kg) that was self-administered prior to naloxone challenge [F (1,20) = 4.72, p < .05]. The predictable stress animals self-administered more fentanyl (mean dosage = 0.24 mg/kg) than did the unpredictable stress animals (mean dosage = 0.17 mg/kg). Further, there was a main effect for sex for the amount of fentanyl that was self-administered prior to naloxone challenge [F (1,20) = 13.78, p < .05]. The females self-administered nearly twice as much fentanyl (mean dosage = 0.26 mg/kg) as did the males (mean dosage = 0.15 mg/kg). There were no significant differences between the unpredictable and predictable groups for withdrawal scores. However, there was a main effect for sex for withdrawal scores following naloxone challenge [F (1,20) = 5.77, p < .05]. Males exhibited more withdrawal behaviors than did the females regardless of stressor condition. A regression analysis with withdrawal score as the dependent variable and sex of the subject as the predictor variable was significant [r^2 = .20, F (1,22) = 5.43, p < .05], indicating that sex was the important determining factor for severity of opioid withdrawal. Specifically, being male was associated with higher withdrawal scores.

Extinction

Figure 9 presents lever responding for water during the first stress phase

of extinction. When water was substituted for the fentanyl solution, there was a significant decrease in lever responding on the operative lever [F (12,240) = 21.4, p < .05] over the first 13 days. In addition, there was significant sex by time interaction [F (12,240) = 2.36, p < .05] and a significant group by time interaction [F (12,240) = 6.24, p < .05], indicating a differential extinction curve among the four experimental groups. Specifically, females took longer than did the males to extinguish lever responding and the predictable stress group took longer than did the unpredictable stress group to decrease lever responding for water.

Relapse

Similar to initiation and testing, fentanyl SA during the no-stress and the stress conditions of the relapse phase [F (1,20)=23.76, p < .05 and F (1,20)=13.67, p < .05, respectively] was significantly greater among females than among males. A repeated measures ANOVA comparing fentanyl SA during the first stress phase of testing to fentanyl SA during the stress phase of relapse revealed a significant group by time interaction [F (1,20)=8.12, p < .05] (see Figure 10). Specifically, animals exposed to predictable stress self-administered significantly less fentanyl during relapse stressor exposure than they did during the testing phase of the experiment. However, animals exposed to unpredictable stress self-administered similar amounts of fentanyl during relapse as they did during the testing phase. There were no differences in drug SA during relapse between the predictable and the unpredictable stress groups under no-stress and stress test conditions.

During relapse, fentanyl SA under no-stress decreased when stress was

reintroduced [$F_{(1,20)}$ = 11.04, $p < .05$]. In addition there was a significant main effect for sex during the no-stress phase of the relapse phase [$F_{(1,20)}$ = 19.43, $p < .05$]. The females self-administered higher amounts of fentanyl than did the males.

Biochemical Assessment

To evaluate whether or not female and male rats in both predictable and unpredictable stress conditions exhibited greater biochemical stress responses (i.e., plasma corticosterone) than did their non-stressed female and male counterparts, groups were collapsed across stress conditions to form a stress group (N=24) that was then compared with the non-stressed control group (N=4).

Unpredictable or predictable stress resulted in plasma corticosterone levels that were significantly higher than plasma corticosterone levels in the non-stress animals [$F_{(1,24)}$ = 12.04, $p < .05$] (see Figure 11), validating the stress manipulation as effective. In addition, overall plasma levels of corticosterone were higher in the females than they were in the males [$F_{(1,24)}$ = 16.48, $p < .05$], consistent with previous reports (Brown, Wood, & Grunberg, 1994; Kant et al., 1983). There were no significant differences between predictable and unpredictable stress groups on biochemical markers of stress.

A regression analysis with plasma corticosterone values as the predictor variable and fentanyl SA during the first stress phase of the testing phase as the dependent variable revealed a significant correlation [r^2 = .34, $F_{(1,22)}$ = 11.61, $p < .05$]. In other words, greater plasma corticosterone levels were positively correlated with fentanyl self-administration.

CONFIRMATION OF MAJOR HYPOTHESES

Hypothesis 1. The hypothesis that rats in the predictable stress condition would self-administer less fentanyl than would rats in the unpredictable stress condition during the beginning of the *Initiation Phase* of the experiment was not confirmed. Specifically, no main effects in fentanyl self-administration were found for stressor condition through day 27 of initiation.

Hypothesis 2. The hypothesis that rats in the predictable stress condition would SA significantly more fentanyl than would rats in the unpredictable stress condition throughout the *Testing Phase* of the experiment was confirmed.

Hypothesis 3. The consistent finding that the majority of female rats, regardless of stressor condition, self-administered significantly more fentanyl than did male rats in either stressor condition disconfirmed Hypothesis 3.

Hypothesis 4. Fentanyl SA did not significantly differ between stress and no-stress test conditions, disconfirming Hypothesis 4.

Hypothesis 5. The finding that subjects that had self-administered fentanyl displayed significantly greater withdrawal behaviors than did animals that did not have prior access to the fentanyl solution confirmed Hypothesis 5.

Hypothesis 6. There were no significant differences between the two stress groups for withdrawal scores following naloxone challenge, confirming Hypothesis 6.

Hypothesis 7. There were no significant differences between predictable and unpredictable stress groups on biochemical markers (i.e., plasma corticosterone) of stress, disconfirming Hypothesis 7.

25

DISCUSSION

This experiment was designed to extend recent findings that have found a causal relationship between stress and opiate self-administration by rats. Using an operant paradigm, female and male rats were tested for drug SA with and without predictable or unpredictable acute and repeated stress. Consistent with earlier behavioral, immunological, and physiological reports (Abbott *et al.*, 1984; Arthur, 1986; Glass & Singer, 1972; McKinnon *et al.*, 1989), predictability had a different effect on drug SA depending on whether the stressor was acute or chronic. Specifically, predictability of the stressor increased fentanyl SA with repeated exposure to the stressor, especially for female subjects. These increased effects persisted whenever the fentanyl solution was available. Further, predictability of the stressor lengthened the duration of the extinction process. This increase in fentanyl consumption and delayed extinction of drug-seeking behavior by the animals exposed to a repeated, acute stressor could be a result of : (1) sensitization to the stressor, or (2) habituation to the effects of the fentanyl.

Further, drug SA by the predictability stress group during the testing phase of the experiment was attenuated during the relapse phase of the experiment. Despite a significantly higher amount of drug SA in the predictably stressed animals during the testing phase of the study, animals in this group self-administered similar amounts of the fentanyl solution during the relapse phase as did animals in the unpredictable stress group. This comparable drug SA may explain the unpredicted finding of similar plasma corticosterone levels, a

26

biochemical marker of stress, in the predictable and unpredictable stress groups at the end of the experiment.

In addition to the predictability effects on drug SA, there also were sex differences in fentanyl consumption. Specifically, female rats self-administered significantly more fentanyl than did male rats, with and without stress, and regardless of whether the stress was predictable or unpredictable. This sex difference began on the third day of drug SA and persisted throughout the study. However, females displayed significantly less withdrawal in response to naloxone challenge than did males. This finding may indicate that females are less sensitive to the effects of opioid agonists and opioid antagonists.

The fact that predictability particularly affected female subjects may suggest that females: (1) developed greater sensitization to the stressor; (2) had greater drug habituation; or (3) were more sensitive to the drug-predictable stress interaction. This finding is consistent with reports that there are sex differences in the effects of physical (Kant *et al.*, 1983) and social stressors (Brown, Wood, & Grunberg, 1994) on stress responses. Therefore, there may be a sex difference in the contribution of the environment and pharmacologic factors to opioid use and abuse. If the present results hold with humans, then females may be less sensitive to drug withdrawal effects, but more sensitive to stress-induced drug taking. Future studies should directly examine these possibilities.

If the present findings can be extended to a clinical setting, then these results suggest that women and men may require differential approaches to addiction treatment. Because males in the current study showed higher

withdrawal responses despite lower opioid SA, treatment for men might focus on pharmacologic replacement therapies, such as methadone maintenance programs. The finding that the females in the present study were more sensitive to psychological variables suggests that females may require treatments that focus on psychological factors that influence drug-taking behavior and that may increase the likelihood of drug relapse such as stress and environmental cues. Clearly, these important clinical implications require direct, empirical assessment.

TABLES

Table 1. Schedule of reinforcement for progressive ratio with a dwell of 2.

Responses (#)	Reinforcers (#)	Fixed-Ratio Schedule
1	1	1
2	2	1
4	3	2
6	4	2
9	5	3
12	6	3
16	7	4
20	8	4
25	9	5
30	10	5
36	11	6
42	12	6
49	13	7
56	14	7
64	15	8
72	16	8
81	17	9
90	18	9
100	19	10
110	20	10
121	21	11
132	22	11
144	23	12
156,	24,	12,
..., 659	..., 50	...,25

Table 2. Amount of fentanyl self-administered during first 13 days of initiation by sex.

Initiation Day	F[1,20]	* = p<.05
1		n.s.
2		n.s.
3	13.86	*
4	15.28	*
5	16.18	*
6	8.64	*
7	14.19	*
8	18.26	*
9	27.83	*
10	23.31	*
11	21.82	*
12	23.48	*
13	11.76	*

FIGURES

FENTANYL

$$CH_3CH_2CON - \underset{\underset{C_6H_5}{|}}{} \hexagon \; N - CH_2CH_2 - C_6H_5$$
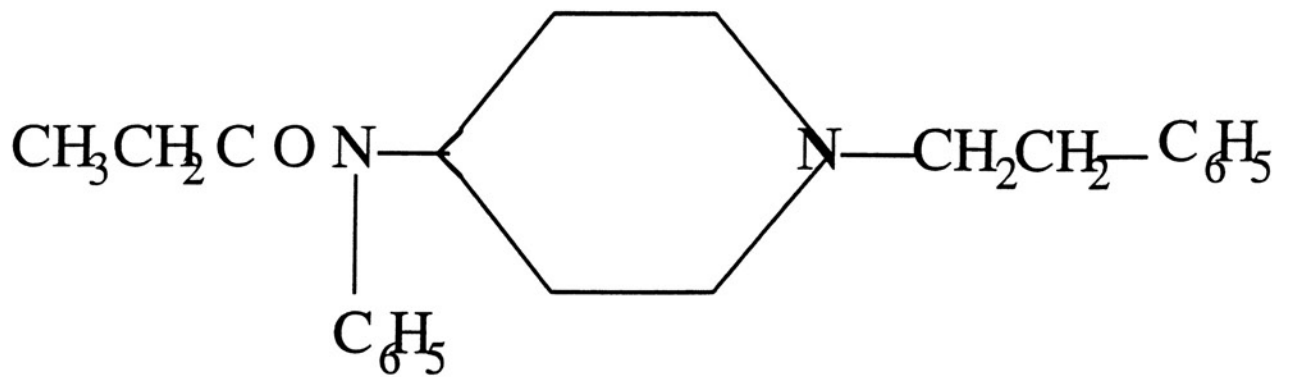
Figure 1. Chemical structure of fentanyl

Training
Phase (15 days)

Initiation
Phase (28 days)

Testing Phase (Four 5 day cycles for a total of 20 days)
·free access to water in the home cage
·PR(2) schedule of reinforcement

STRESS + DRUG   NO-STRESS + DRUG   STRESS(2) + DRUG   NO-STRESS(2) + DRUG

Days 50-54   55-59   60-64   65-69
      ↑
Naloxone Challenge

Extinction Phase (20 days)   **Relapse Phase** (9 days)
·free access to water in the home cage   ·free access to water in the home cage
·PR(2) schedule of reinforcement   ·PR(2) schedule of reinforcement

STRESS + H$_2$O   NO-STRESS + H$_2$O   STRESS(2) + H$_2$O NO-STRESS + DRUG   STRESS(2) + DRUG

Days 70-82   83-87   88-89   ↑ 90-93   94-98
                                            7 day
                                         rest period

Rest Phase (3 weeks)   **Stressor Validation** (5 days)
·No experimental manipulations   ·animals exposed to stressor (10 min) without drug
                                                  ·animals sacrificed on last day after exposure to stressor

---

Days 99-120   121-125

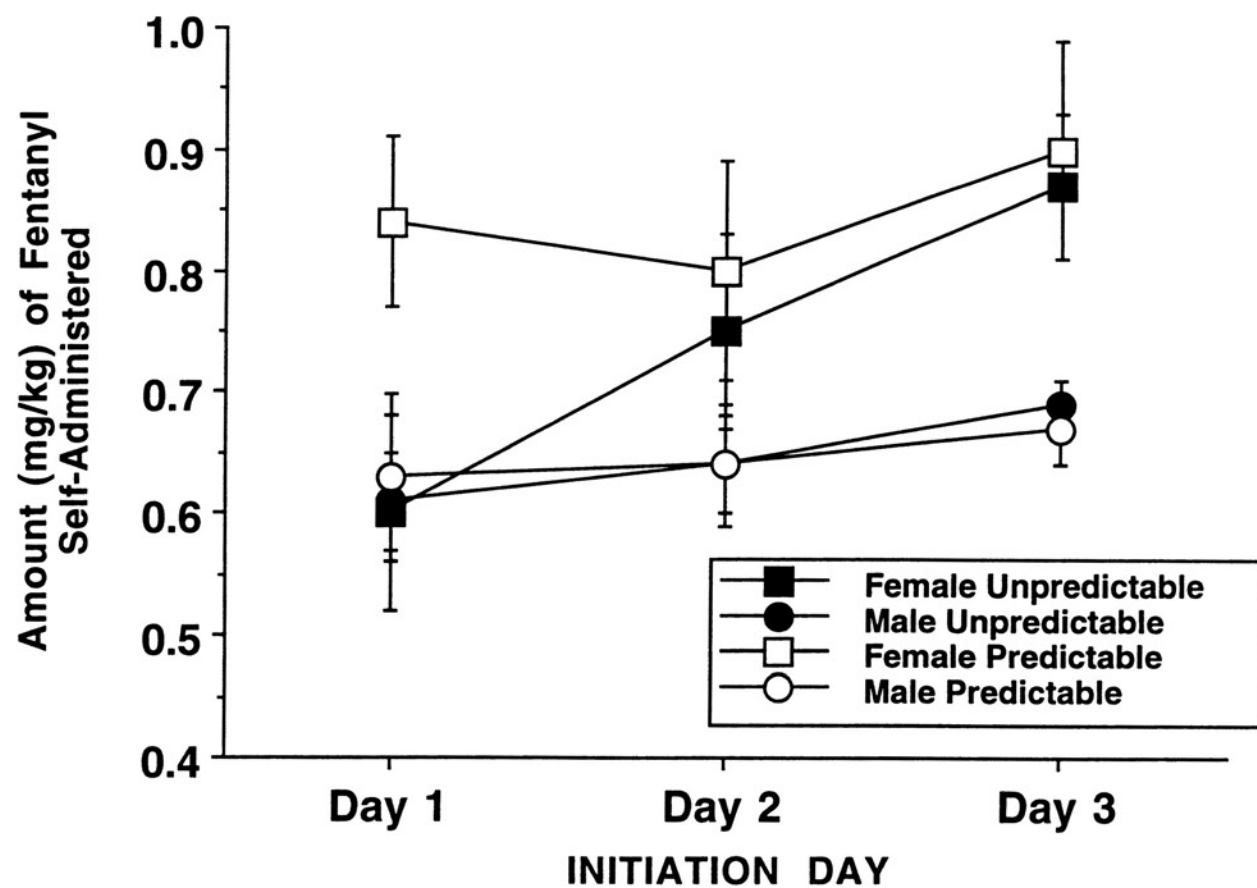Figure 2. Experiment timeline

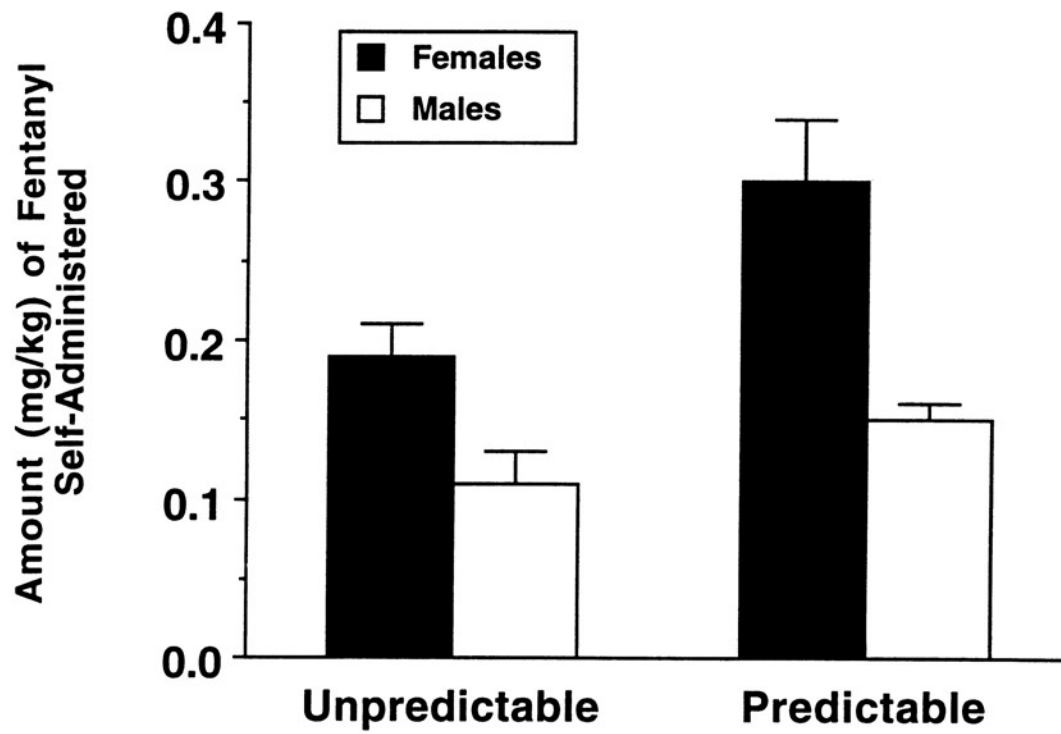Figure 3. Drug self-administration during initiation (means and standard errors)

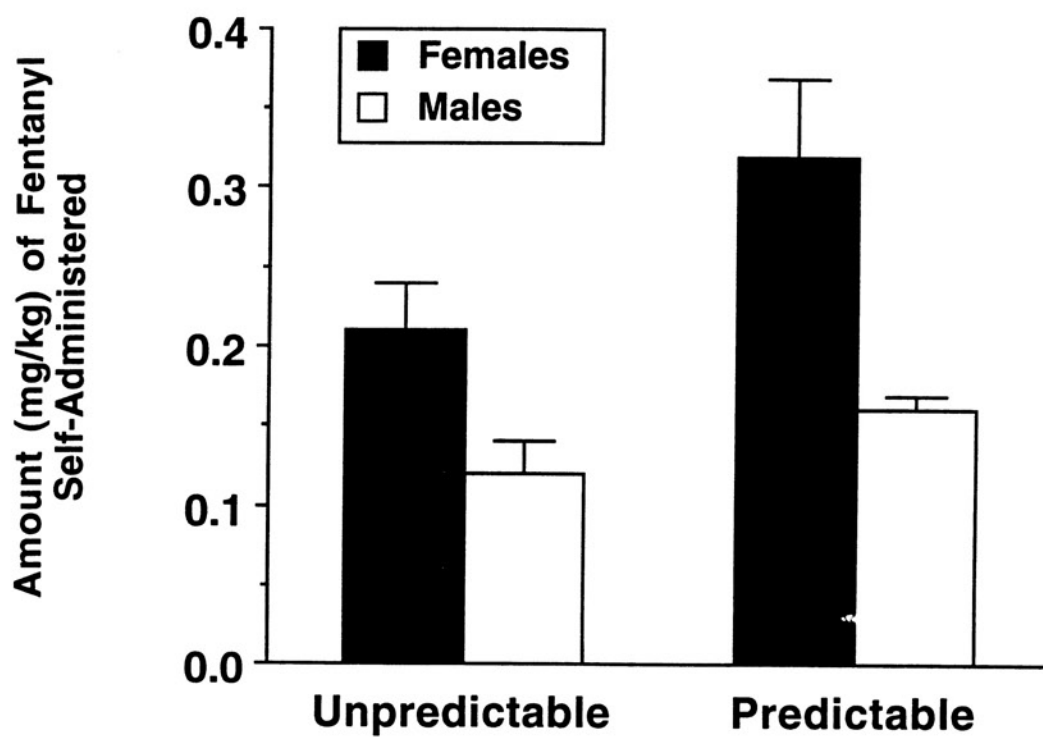Figure 4. Drug self-administration during first stress phase (means and standard errors)

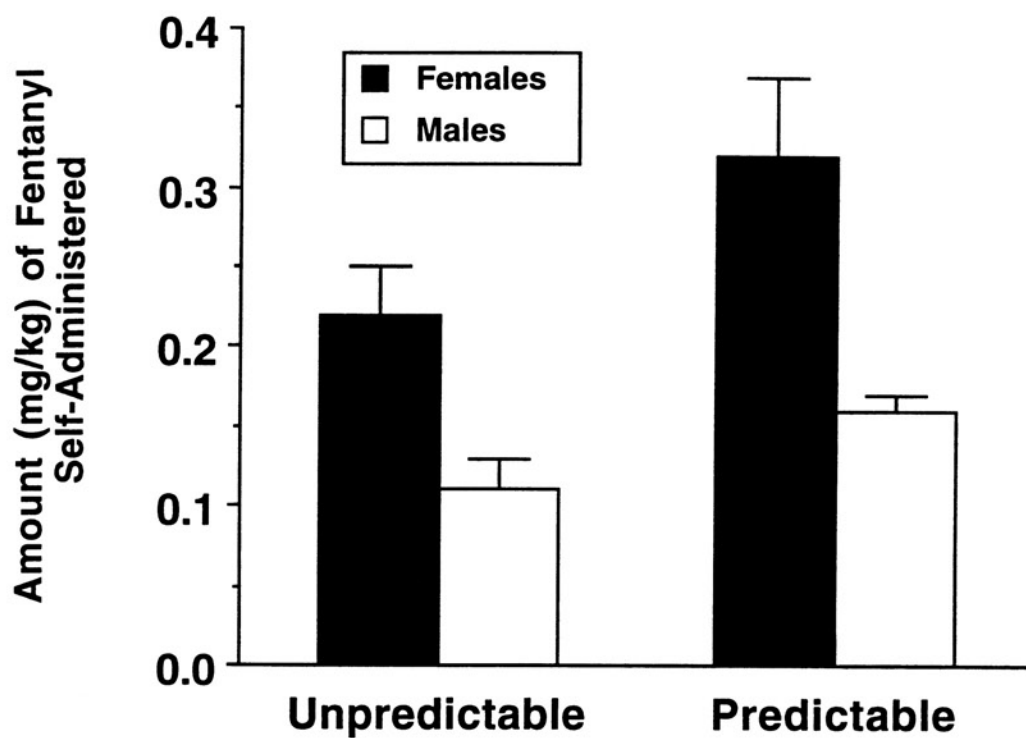Figure 5. Drug self-administration during first no-stress phase (means and standard errors)

Figure 6. Drug self-administration during second stress phase (means and standard errors)
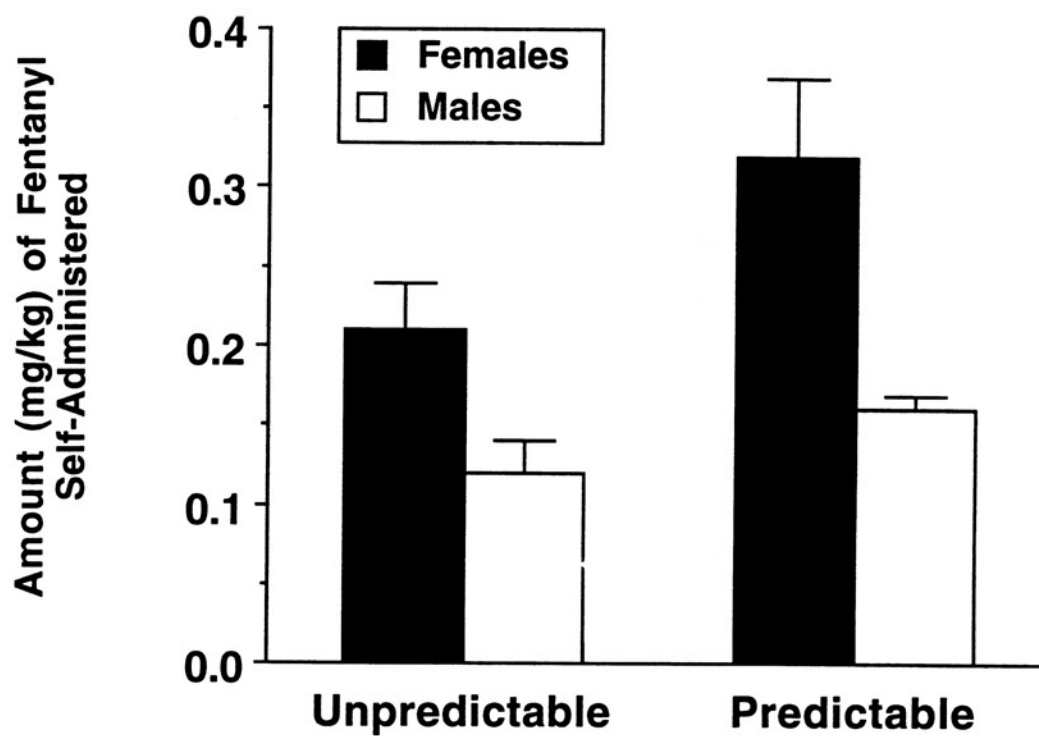
Figure 7. Drug self-administration during second no-stress phase (means and standard errors)
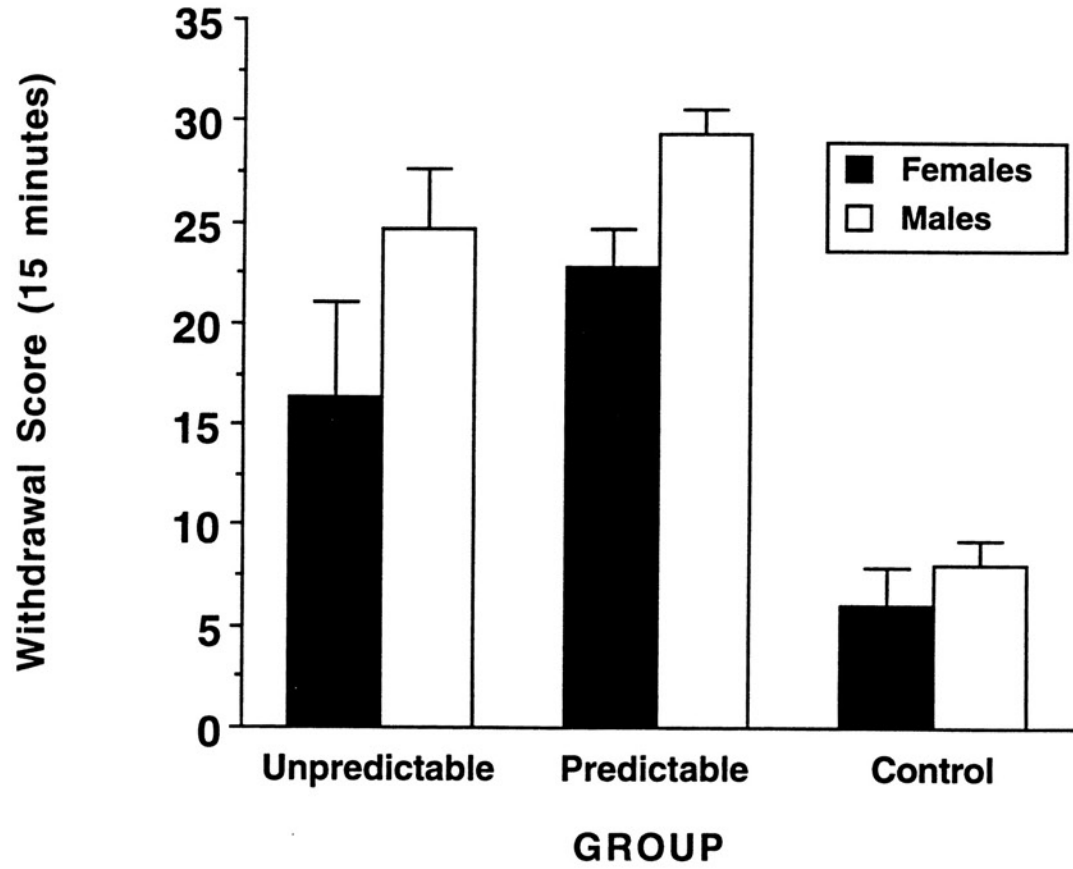
Figure 8. Withdrawal scores following naloxone (1.5 mg/kg) challenge (means and standard errors)
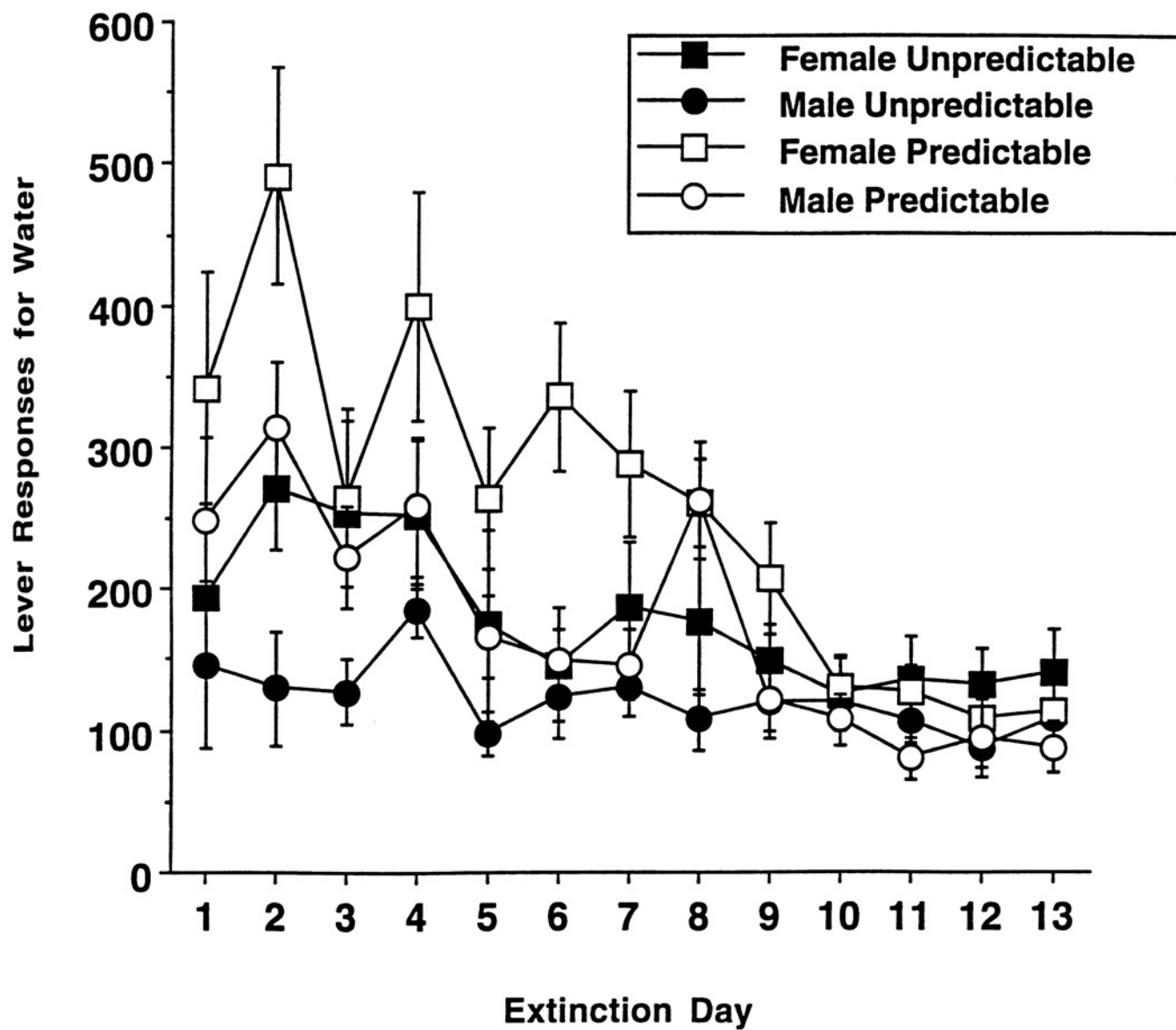
Figure 9. Lever responding (progressive ratio) for water during the stress phase of extinction (means and standard errors)
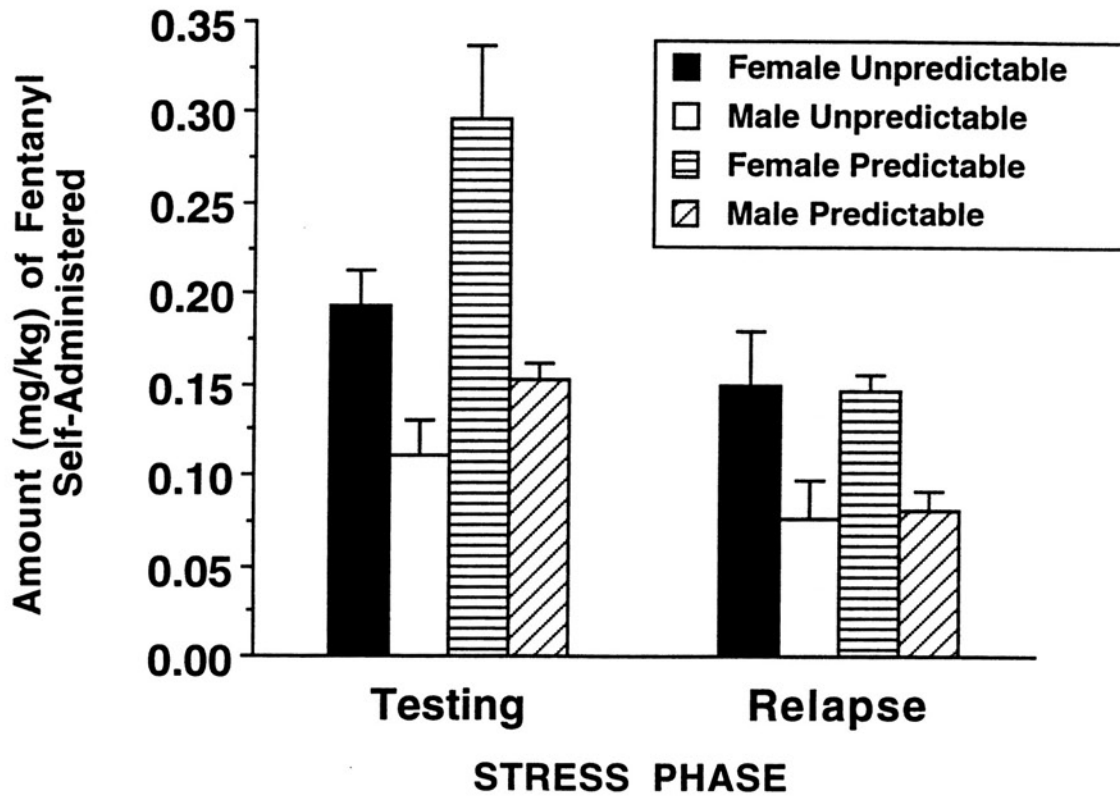
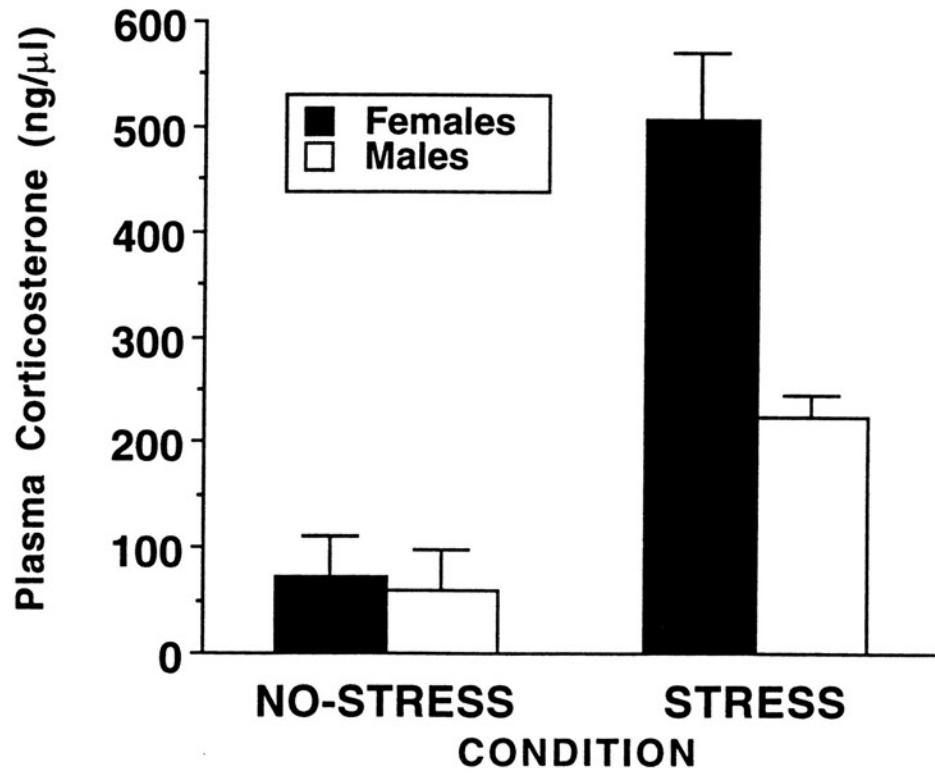Figure 10. Drug self-administration during testing and relapse stress conditions (means and standard errors)

Figure 11. Plasma corticosterone (ng/µl) responses (means and standard errors)

APPENDIX I

Withdrawal Observation Data Sheet

WITHDRAWAL SYMPTOMS OBSERVATION DATA SHEET

Body Weight: Pre_____ Post_____ Change_____

Naloxone Dosage:_____ml _____mg/kg

1. **Wet-dog shakes** (# of times):

_____

2. **Diarrhea** (# of times):

_____

3. **Mouthing and teeth chattering** (# of times):

_____

4. **Ptosis** (# of times):

_____

5. **Excessive grooming** (# of times):

_____

6. **Abnormal posture** (# of times):

_____

Observer Initials:_____ Date:_____ Time:_____

Subject#:_____ **TOTAL SCORE**:_____

REFERENCES

Abbott, B.B., Schoen, L.S., & Badia, P. (1984). Predictable and unpredictable shock: Behavioral measures of aversion and psychological measures of stress. Psychological Bulletin, 96(1), 45-71.

Arthur, A.Z. (1986). Stress of predictable and unpredictable shock. Psychological Bulletin, 100(3), 379-383.

Brown, K.J., Wood, L., & Grunberg, N.E. (1994 August). Crowding stresses male but calms female rats. Presented at American Psychological Association, Los Angeles, California.

Davis, H., & Levine, S. (1982). Predictability, control, and the pituitary-adrenal response in rats. Journal of Comparative and Physiological Psychology, 96(3), 393-404.

DeBoer, S.F., van der Gugten, J., & Slangen, J.L. (1989). Plasma catecholamine responses to predictable and unpredictable noise stress in rats. Physiology and Behavior, 45, 787-795.

Depoortere, R.Y., Li, D.H., Lane, J.D., & Emmett-Oglesby, M.W. (1993). Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacology Biochemistry and Behavior, 45, 539-548.

Dib, B. (1985). A study of intrathecal self-injection of morphine by rats, and the difficulties entailed. Pain, 23, 177-185.

Dib, B., & Duclaux, R. (1982). Intracerebroventricular self-injection of morphine in response to pain in rats. Pain, 13, 395-406.

Glass, D., & Singer, J. (1972). Urban Stress. New York: Academic Press.

Grunberg, N.E., & Baum, A. (1985). Biological commonalities of stress and substance abuse. In Shiffman, S. & Wills, T. A. (Eds.). Coping and Substance Abuse. New York: Academic Press, Inc.

Grunberg, N.E., Winders, S.E., & Wewers, M.E. (1991). Gender differences in tobacco use. Health Psychology, 10, 143-153.

Hall, S.M., Havassy, B.E., & Wasserman, D.A. (1990). Commitment to abstinence and acute stress in relapse to alcohol, opiates and nicotine. Journal of Consulting and Clinical Psychology, 58, 175-181.

Jaffe, J.H., & Martin, W.R. (1990). Opioid analgesics and antagonists. In A.G. Gilman, T.W. Rall, A.S. Nies, & P. Taylor (Eds.), Goodman and Gilman's: The pharmacological basis of therapeutics (8th ed.), pp. 485-521. New York: McGraw Hill.

Kant, G.J., Lenox, R.H., Bunnell, B.N., Mougey, E.H., Pennington, L.L., & Meyerhoff, J. L. (1983). Comparison of the stress response in male and female rats: Pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. Psychoneuroendocrinology, 8, 421-428.

Klein, L.C., Shaham, Y., Alvares, K., & Grunberg, N.E. (1993 August). Effect of stress on oral fentanyl consumption in female rats. Presented at American Psychological Association Conference, Toronto, Canada.

Kosten, T.R., Rounsaville, B.J., & Kleber, H.D. (1986). A 2.5 year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. Archives of General Psychiatry, 43, 733-739.

Lex, B.W. (1991). Some gender differences in alcohol and polysubstance users. Health Psychology, 10(2), 121-132.

Linseman, M.A. (1977). Naloxone-precipitated withdrawal as a function of the morphine-naloxone interval. Psychopharmacology, 54, 159-164.

Miller, R.R., & Matzel, L.D. (1989). Contingency and relative associative strength. In S.B. Klein & R.R. Mower (Eds.), Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory (pp. 61-84). New Jersey: Lawrence Erlbaum Associates.

McKinnon, W., Weisse, C.S., Reynolds, C.P., Bowles, C.A., & Baum, A. (1989). Chronic stress, leukocyte subpopulations, and humoral response to latent viruses. Health Psychology, 8, 389-402.

O'Doherty, F. (1991). Is drug use a response to stress? Drug and Alcohol Dependence, 29, 97-106.

O'Doherty, F., & Davies, B.J. (1987). Life events and addiction: A critical review. British Journal of Addiction, 82, 127-137.

Pavlov, I. (1906). The conditioned reflex. In W.A. Hillix & M.H. Marz (Eds.), Systems and theories in psychology: A reader (99-106). St. Paul: West Publishers.

Rescorla, R.A. (1966). Predictability and number of pairings in Pavlovian fear conditioning. Psychonomic Science, 4, 383-384.

Rescorla, R.A. (1967). Pavlovian conditioning and its proper control procedures. Psychological Review, 74, 71-80.

Roberts, D.C.S., Bennett, S.A.L., & Vickers, G.J. (1989). The estrous cycle affects cocaine self-administration on a progressive-ratio schedule in rats. Psychopharmacology, 98, 408-411.

Schulz, R. (1976). Effects of control and predictability on the physical and psychological well-being of the institutionalized aged. Journal of Personality and Social Psychology, 33, 563-573.

Seggie, J.A., & Brown, G.M. (1975). Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat following handling or exposure to novel environment. Canadian Journal of Physiology and Pharmacology, 53, 629-637.

Shaham, Y. (1993). Immobilization stress-induced oral opioid self-administration in rats: Role of conditioning factors and the effect of stress on "relapse" to opioid drugs. Psychopharmacology, 111, 477-485.

Shaham, Y., Alvares, K., Nespor, S.M., & Grunberg, N.E. (1992). Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacology Biochemistry and Behavior, 41, 615-619.

Shaham, Y., Klein, L.C., Alvares, K., & Grunberg, N.E. (1993). Effect of stress on oral fentanyl consumption in rats in an operant self-administration paradigm. Pharmacology Biochemistry and Behavior, 46, 315-322.

Shiffman, S., & Wills, T.A. (1985). Coping and substance abuse. Orlando: Academic Press.

Staub, E., Tursky, B., & Schwartz, G.E. (1971). Self-control and predictability: Their effects on reactions to aversive stimuli. Journal of Personality and Social Psychology, 18(2), 157-162.

Taylor, J., Harris, N., & Vogel, W.H. (1990). Voluntary alcohol and cocaine consumption in "low" and "high" stress plasma catecholamine responding rats. Pharmacology Biochemistry and Behavior, 37, 359-363.

Tatham, T.A., & Zurn, K.G. (1989). The MED-PC experimental apparatus programming system. Behavior Research Methods: Instrumentation Computations, 21, 294-302.

Weiss, J.M. (1970). Somatic effects of predictable and unpredictable shock. Psychosomatic Medicine, 32, 397-40.

Whitehead, C.C. (1974). Methadone pseudowithdrawal syndrome: Paradigm for a psychopharmacological model of opiate addiction. <u>Psychosomatic Medicine, 36,</u> 189-198.